

Cations Mediate B-DNA Conformational Heterogeneity

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Beamline(s): X26C

Introduction: We demonstrate that DNA conformation is sensitive to cationic environment. We describe a high-resolution (1.2 Å) potassium form of CGCGAATTCGCG, determined from crystals grown in the presence of spermine and magnesium, along with potassium. The structure was refined with anisotropic displacement-factors by SHELX to an R-factor of 13.9%. A comparison of this structure with others, reveals that the conformation of CGCGAATTCGCG varies in direct response to cation type and position. The DNA conformation in the presence of excess magnesium differs from the conformation in the presence of excess spermine. Divalent cations near the minor groove sequester into the lip, which is the region between opposing phosphate groups. Minor groove width is sensitive to, and can be predicted by, cation positions. It appears that minor groove narrowing is facilitated by interactions of cations with opposing phosphate groups.

Methods and Materials: We describe the crystallization, data collection and reduction, and refinement of a 1.2 Å resolution CGCGAATTCGCG structure obtained from a crystal grown in the presence of potassium along with spermine and magnesium. We have previously described a 1.4 Å resolution CGCGAATTCGCG structure (NDB entry BDL084) (Shui et al., 1998a) obtained from a crystal grown in the presence of sodium, spermine, and magnesium. We have also described 1.75 Å resolution CGCGAATTCGCG structure (NDB entry BD0005) (Shui et al., 1998b) obtained from a crystal grown in the presence of potassium, spermine, and magnesium, and a 1.8 Å resolution CGCGAATTCGCG structure (NDB entry BD0029) (Woods et al., 2000) obtained from a crystal grown in the presence of cesium, spermine, and magnesium. For the high-resolution spermine/potassium structure described here, the ammonium salt of reverse-phase HPLC purified d(CGCGAATTCGCG) was purchased from the Midland Certified Reagent Company (Midland, TX). Crystals were grown at 22 °C from sitting drops that initially contained 1.0 mM d(CGCGAATTCGCG), 19 mM potassium cacodylate (pH 6.5), 10 mM magnesium chloride, 8.9 mM spermine tetrahydrochloride, and 4.8% 2-methyl-2,4-pentanediol (MPD). The droplets were equilibrated by vapor diffusion against a reservoir of 50% MPD. These crystallization conditions are similar to those of the previous 1.75 Å resolution spermine/potassium structure of CGCGAATTCGCG. Orthorhombic (P2₁2₁2₁) crystals appeared within several days. The crystal chosen for data collection was 1.1 x 0.4 x 0.4 mm³.

Data Collection and Reduction, and Structure Refinement. Intensity data were collected on Beamline X26C at the National Synchrotron Light Source, Brookhaven National Laboratory. Crystals were flash frozen by immersion in a stream of 175K nitrogen gas (Oxford Cryosystems). Intensities were recorded on a Quantum 4 CCD detector at a wavelength of 1.1 Å. Data were processed with the software package HKL (Otwinowski & Minor, 1997). The quality of the final model, which was refined against 19,368 unique reflections with SHELX-97 (Sheldrick, 1997) is high as inferred from electron density maps, displacement ellipsoids, R-factor (13.9%), R-Free (21.5%).

Conclusions: Various metal ions specifically stabilize Z-DN (Pohl & Jovin, 1972; van de Sande et al., 1982), G-quartets (Williamson et al., 1989; Smirnov & Shafer, 2000), and other non-canonical conformations (Wohlrab & Wells, 1989). Recently it has been demonstrated that divalent zinc, nickel and cobalt can intercalate in DNA, substituting divalent cations for imino protons, to form 'M-DNA' (Aich et al., 1999). The variation of DNA conformation with changing cation positions, while sequence and lattice are fixed, demonstrated here indicates that specific interactions between DNA and surrounding cations contribute to conformational heterogeneity of B-form CGCGAATTCGCG. The possibility of observing additional ionic forms in additional conformational states is high. The two forms described here appear to be discrete and exclusive in that either spermine plus one magnesium ion or multiple magnesium ions are observed. We anticipate that in crystals, the effects of ions on DNA conformation variation would be attenuated in comparison to those in the solution state. In the crystalline environment (i) intermolecular electrostatic forces would compete with intramolecular forces, (ii) sequence-specific effects on cation distributions would be perturbed by lattice effects, and (iii) steric restraints would dampen the structural consequences of electrostatic forces. These considerations support the proposal by Crothers that the A-tract conformation of CGCGAATTCGCG might be different in crystals and dilute solution (Haran et al., 1994). If electrostatic forces are significant, deviations of solution from crystalline behavior should be least pronounced for short-range phenomena such as groove width variation, and most pronounced for long-range phenomena such as DNA bending. In fact the cation-related perturbations observed here, and by Rees and coworkers (Kielkopf et al., 2000), are most pronounced for local phenomenon. Solution-like A-tract bending has not been observed thus far in oligonucleotide crystals. A combination of technical advances should allow us to significantly increase the detail of the 'cation map' surrounding DNA. The quality of crystals grown in a host of laboratories continues to increase. The quality and availability of synchrotron radiation sources and fast readout detectors continues to increase. Full matrix refinement with application of anisotropic displacement-factors is now routinely applicable to large molecules, if data quality is sufficient. Substitution of potassium with thallium may allow construction of complete 'monovalent cation maps' surrounding DNA and RNA.